

Efficacy of Tamra Sindoor Prepared with Ashta Samskarita Parada over Cervical Cancer a Cell Line Study

Dr. Kusum Kumari¹, Dr. Pradeep Agnihotri², Dr. K.M Jaggal³

¹Post Graduate Scholar, ²Professor, ³Professor and HOD,
^{1,2,3}Department of PG Studies in Rasashastra and Bhaishajya Kalpana,
Ayurveda Mahavidyalaya Hubballi, Karnataka, India

ABSTRACT

The metal and mineral based drugs have been prescribed in *Ayurveda* for ancient period of time to treat various chronic diseases and also different types of Cancer. Now a day's Cancer has become the biggest challenge to the scientific community over the world. According to global consideration- among all cancer, cervical cancer is the 2nd most common and most lethal malignancy in women worldwide and also in gynecological concern. *Tamra Sindoor* is one of the *Kupipakwa Rasayana* preparations and indicated in *Raktajanya Vikaras, Vata-Kapha Pradhana Mamsarbuda*. In present study it is prepared with *Ashta Samskarita Parada*. *Ashta Samskarita Parada* is free from *Doshas* and also makes it easily digestible, absorbable, and easy for assimilation by the tissue and having *Sarvarogahara*, and *Rasayana* properties. So because of these properties, to fortify *Tamra Sindoor*, *Ashta Samskarita Parada* was used to produce augmented anticancerous effect. So the proper scientific Validation of fortify *Tamra Sindoor* has become one of the focused research work of new drug against Cervical Cancer. Fortify *Tamra Sindoor* was tested for Anti-cancerous activity over Cervical Cancer with different concentration. Drug shows maximum lysis of 55.7% with higher dose of 1000ug/ml.

KEYWORDS: *Ashta Samskarita Parada, Cervical Cancer, Cell Line Study, Kupipakwa Rasayana, Tamra Sindoor*

INTRODUCTION

Now a day's Cancer has become the biggest challenge to the scientific community over the world. According to global consideration- among all Cancer, Cervical Cancer is the 2nd most common and most lethal malignancy in women worldwide and also in gynecological concern. Nearly 500,000 cases of Cervical Cancer are expected worldwide with approximately 240,000 deaths annually¹. The HELA cell was the first human cell line was established in a Baltimore laboratory over 50 years ago by George Gey. This cell line was HELA named after Henrietta Lacks, the lady from whom the cell line was derived, who had cervical carcinoma. These cells are a suitable transfection host. There are many strains of HELA cells as they continue to mutate in cell cultures². The Cell culture and cell lines have assumed an important role in studying physiological, pathological and differentiation processes of specific cells. It allows the examination of stepwise alterations

in the structure, biology, and genetic makeup of cell under controlled environments.

The modern treatment is effective but a very painful and the adverse effect of chemo-radiations therapy is harmful to the body. These treatment modalities are very costly and need continuous medical intervention and this is not affordable for everyone. Keeping a view on all these thoughts where *Ayurveda* lines of treatment found to be safer and cost-effective in palliative management. In classical *Rasaoushadhies* have their own importance and major role in day-to-day practice because of their quick onset of action and higher efficacy in a smaller dosage. In classical mentioned four varieties of *Rasa Kalpas* viz.- *Kharaliya Rasayana, Parpati Rasayana, Kupipakwa Rasayana*, and *Pottali Rasayana*. Among four *Rasa Kalpas*, *Kupipakwa Rasayana* is the unique method of preparation and it deserves special importance

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because of its minimal dosage, maximum effect, long lasting-potency, and synergistic effects in the body. And is very potent in eliminating not only acute disease but also chronic diseases and acts as rejuvenating agents³.

Tamra Sindoor is one of the *Kupipakwa Rasayana* and indicated in *Raktajanya Vikaras* and *Vata-Kapha Pradhana Mamsarbuda*. Due to the *Lekhana Guna* of *Tamra Sindoor* useful in *Kaphaja-Granthi*, *Gulma*

Roga, and best in *Kitanuhara* property. The *Tamra Sindoor* contains *Shuddha Parada* which is *Sarvarogahara*, *Shuddha Gandhaka* having *Mrutyu-Jaranashaka*, and *Shuddha Tamra* is *Param Lekhana* properties. The medicine is prepared out of *Astha Samskarita Parada* and other drugs by the *Kupipakwa* method which is having more potent and anticancer effect were selected and compared over Cervical Cancer through cell line study.

MATERIAL AND METHOD

PREPARATION OF ASTA SAMSKARITA PARADA⁴:-

- *Ashta Samskaras* of *Parada* was done as per the *Rasa Hridaya Tantra*.
- The total quantity of *Parada* taken for *Asta Samskara*- 500gms.
- Weight of *Parada* obtained after *Ashta Samskara* - 350gms.
- 30% of loss was found after *Ashta Samskaras*.

PREPARATION OF TAMRA SINDOOR⁵:-

The *Tamra Sindoor* is prepared by the Method of *Bhairdhooma Kupipakwa*

1. *Asta Samskarita Parada* and *Shodhita Gandhaka* triturated and prepared *Kajjali*.
2. This *Kajjali* filled in *Kacha Kupi* then pieces of *Shodhita Tamra* Added in it.
3. This *Kacha Kupi* is placed in *Valuka Yantra* and Subjected for *Kramagni* for 36 Hours.
4. After *Swangsheeta* the *Kupi* is taken out, fortified *Tamra Sindoor* collected at *Kantha Bhaga* and stored.

Table No 1

Total quantity of Ingredients			Weight of <i>Tamra Sindoor</i>	Weight of <i>Talastha Tamra Bhasma</i>	Total weight	Wt. loss	Yield
Total wt. of <i>Kajjali</i>	Total wt. of <i>Shu. Tamra</i>	Total quantity of ingredients					
238gms	60gms	298gms	177gms	99gms	276gms	22gms	60%

CELL LINE STUDY⁶

Principle:-The MTT Assay is a colorimetric assay for assessing cell metabolic activity. These enzymes are capable of reducing the tetrazolium dye MTT 3 (4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (yellow dye) to its insoluble formazan, which has a purple color. Tetrazolium dye assays can be used to measure cytotoxicity (loss of viable cells) or cytostatic activity (shift from proliferation to quiescence) of potential medicinal agents and toxic materials. MTT assays are usually done in the dark since the MTT reagent is sensitive.

MTT solution preparation:-5 mg in 1 ml of Phosphate Buffer Saline (PBS – pH 7.4).

Methodology:-In vitro growth inhibition effect of test compound was assessed by colorimetric or spectrophotometric determination of conversion of MTT into “Formazan blue” by living cells.

Day1:-1 × 10⁵ cells/ml cell suspension was seeded into each well in a 96 well micro titer plate and final volume was made upto 150 µl by adding DMEM (Dulbecco’s modified egle medium) media and incubated overnight.

Day 2:-Dilutions of the test compounds were prepared in DMEM media.

100µl of the test compounds of different concentrations was added to the wells and incubated for 24 hrs, in presence of 5 % CO₂, at 37⁰C into CO₂ incubator.

Day 3:-After 24 hrs, 20µl of 5 mg/ ml MTT reagent was added to the wells. The plate was kept for 4 hrs incubation in dark place at room temperature. (The plate was covered with aluminum foil, since MTT reagent is photosensitive.)

The supernatant was carefully removed without disturbing the precipitated Formazan crystals and 100 µl of DMSO (Dimethyl sulfoxide) was added to dissolve the crystals formed.

The optical density (OD) was measured at wavelength of 492 nm.

The study was performed in triplicates and the result represents the mean of three readings.

Formula:-

$$\text{Surviving cells (\%)} = \frac{\text{Mean OD of test compound}}{\text{Mean OD of control (untreated cells)}} \times 100$$

Table no.2-Table showing MTT Assay result of *Tamra Sindoor*:

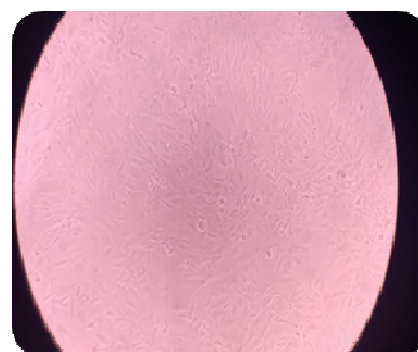
Compound	Concentration (ug/ml)	Cell viability (%)	% of Lysis
<i>Tamra Sindoor</i>	1000	44.3	55.7
	500	49.9	50.1
	250	50.4	49.6
	125	53.9	46.1
	62.5	58.7	41.3
L929 (compatibility)	1000	73	27
	500	75.4	24.6
	250	81.9	18.1
	125	83.2	16.8
	62.4	88.1	11.9

DISCUSSION:-

Tamra Sindoor prepared with *Asta Samskarita Parada* enhances- *Tikshna*, *Rasayana*, *Param Lekhana* properties. So, due to these properties it act as *Gulmahara*, *Arbudahara*, *Granthihara*, and it is very effective in *Raktajanya Vikaras*. The fortify drug *Tamra Sindoor* was tested on Cervical Cancer (HELA cell) in cell line study with five different concentrations viz. 1000ug/ml, 500ug/ml, 250ug/ml, 125ug/ml, and 62.5ug/ml. Fortify *Tamra Sindoor* showed maximum lysis effect with higher dosage 1000ug/ml i.e. 55.7% lysis was observed. The percentage of lysis can be increased by increasing the dose of drug. The fortify drug *Tamra Sindoor* was tested for its compatibility on cell L929 (normal subcutaneous areola adipose cell) with five different concentration viz. 1000ug/ml, 500ug/ml, 250ug/ml, 125ug/ml, 62.5ug/ml. Sample showed the lysis effect on adipose cell. Fortify *Tamra Sindoor* having *Param Lekhana* property it does the lysis of adipose cell.

lysis of 27% with higher dose 1000ug/ml on adipose cell. This imply that *Asta Samskarita Parada* preparation do possess higher therapeutic potency. Hence the study proved that the *Tamra Sindoor* prepared with *Asta Smskarita Parada* has promising Anti-Cancerous activity.

Microscopic structure of *Tamra Sindoor*
Microscopic structure of *Tamra Sindoor* over the HELA cell over L929 cell

**CONCLUSION:-**

Tamra Sindoor is *Kupipakwa Sagandha*, *Bhirdhooma*, and *Kanthastha Rasayana* mainly explained in *Ayurveda Sara Sangraha*. *Tamra Sindoor* acts on *Raktaja Arbuda*, *Granthi*, *Gulma*, *Vata-Kapha Pradhana Mamsarbuda* and *Raktajanya Vikaras*. The fortify *Tamra Sindoor* was tested for Anti-Cancerous activity over Cervical Cancer with different concentration i.e-1000ug/ml, 500ug/ml, 250ug/ml, 125ug/ml and 62.5ug/ml. Sample shows maximum lysis of 55.7% with higher dose of 1000ug/ml. and when fortify *Tamra Sindoor* was tested for its compatibility on cell L929 with five different concentration viz. 1000ug/ml, 500ug/ml, 250ug/ml, 125ug/ml, 62.5ug/ml. Sample showed the

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